

## Thermodynamics

1. For the calculation of equilibrium constant (K<sub>eq</sub>) for PGK in *S. solataricus*, we used the following formula (Formula 1).

$$K_{eq_{PGK}} = \frac{[V_{mfPGK}][K_{PGKATP}][K_{PGK3PG}]}{[V_{mrPGK}][K_{PGKADP}][K_{PGKBPG}]} = \frac{\frac{37.96U}{mg} \times 9.309mM \times 0.567mM}{\frac{17.212U}{mg} \times 0.374mM \times 0.0082mM} = 3793 \quad (1)$$

2. For the calculation of K<sub>eq</sub> for GAPDH in *S. solataricus*, we used the following formula (Formula 1).

$$K_{eq_{GAPDH}} = \frac{[V_{mfGAPDH}][K_{GAPDHADPH}][K_{GAPDHBPG}]}{[V_{mrGAPDH}][K_{GAPDHADP}][K_{GAPDHGAP}][K_{GAPDHPi}]} = \frac{\frac{35.551U}{mg} \times 0.09445mM \times 0.0895mM}{\frac{23.6115U}{mg} \times 0.204mM \times 3.1091mM \times 108.32mM} = 1.85 \times 10^{-4} mM^{-1} \quad (2)$$

3. For K<sub>eq</sub> of PGK in *S. cerevisiae* we used the one in the model the value was 3200.

4. While for K<sub>eq</sub> of GAPDH in *S. cerevisiae*, we calculated it again according to the following formula (3).

$$K_{eq} = \frac{keq}{K_{Pi}} = \frac{0.0054}{0.0015M} = 3.60M^{-1} \quad (3)$$

Where keq 0.0054 is the value in the model, while K<sub>Pi</sub> is 0.0015M.

5. In order to investigate the influence of phosphate for GAPDH, we named standard Gibbs free energy without phosphate as  $\Delta G^{0'}$  and calculated using the following formula (4).

$$\Delta G^{0'} = -RT \ln(K_{eq}) \quad (4)$$

Where in the formula R is gas constant and its value is 8.3kJ<sup>-1</sup>mol<sup>-1</sup>, T is temperature in Kelvin, for calculation  $\Delta G^{0'}$  of *S. solataricus* T value is 347 K; for calculation  $\Delta G^{0''}$  of *S. cerevisiae* T value is 307 K.

The following are the calculations.

$$\Delta G^{0'}(S.s) = -RT \ln(K_{eq}) = -\frac{8.314J}{mol \cdot K} \times 343K \ln\left(\frac{0.18}{M}\right) = \frac{4.9kJ}{mol}$$

$$\Delta G^{0'}(S.c) = -RT \ln(K_{eq}) = -\frac{8.314J}{mol \cdot K} \times 303K \ln\left(\frac{3.6}{M}\right) = -\frac{3.2kJ}{mol}$$

6. The standard Gibbs energy change for GAPDH with influence of phosphate we named as  $\Delta G^{0''}$ , we used the following formula (5) to calculate  $\Delta G^{0''}$ .

$$\Delta G^{0''} = \Delta G^{0'} + RT \ln \frac{1}{Pi} \quad (5)$$

Where Pi is 10mM,100mM separately.

$$\Delta G^{0''}(S.s : Pi = 1mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{4.9kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \ln \left( \frac{1}{0.001} \right) = \frac{24.4kJ}{mol}$$

$$\Delta G^{0''}(S.s : Pi = 10mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{4.9kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \ln \left( \frac{1}{0.01} \right) = \frac{17.9kJ}{mol}$$

$$\Delta G^{0''}(S.s : Pi = 100mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{4.9kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \ln \left( \frac{1}{0.1} \right) = \frac{11.4kJ}{mol}$$

$$\Delta G^{0''}(S.s : Pi = 1M) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{4.9kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \ln \left( \frac{1}{1} \right) = \frac{4.9kJ}{mol}$$

$$\Delta G^{0''}(S.c : Pi = 1mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{-3.2kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 303K \ln \left( \frac{1}{0.001} \right) = \frac{14.2kJ}{mol}$$

$$\Delta G^{0''}(S.c : Pi = 10mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{-3.2kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 303K \ln \left( \frac{1}{0.01} \right) = \frac{8.4kJ}{mol}$$

$$\Delta G^{0''}(S.c : Pi = 100mM) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{-3.2kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \ln \left( \frac{1}{0.1} \right) = \frac{2.6kJ}{mol}$$

$$\Delta G^{0''}(S.c : Pi = 1M) = \Delta G_0' + RT \ln \frac{1}{[Pi]} = \frac{-3.2kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 303K \ln \left( \frac{1}{1} \right) = \frac{-3.2kJ}{mol}$$

7. As phosphate has no influence on the standard Gibbs energy change for PGK, we used the following formula to calculate  $\Delta G^{0''}$  for PGK

$$\Delta G^{0''} = -RT \ln (Keq) \quad (6)$$

$$\Delta G^{0''}(S.s) = -RT \ln (Keq) = -\frac{8.314J}{mol \cdot K} \times 343K \ln (3793) = -\frac{23.5kJ}{mol}$$

$$\Delta G^{0''}(S.c) = -RT \ln (Keq) = -\frac{8.314J}{mol \cdot K} \times 303K \ln (3200) = -\frac{20.3kJ}{mol}$$

8. While for the calculation of  $\Delta G$ , we used the following formula (7).

$$\Delta G = \Delta G^{0''} + RT \ln \frac{[P_1][P_2]}{[S_1][S_2]} \quad (7)$$

Where in the formula [P1] and [P2] represent the product concentrations at steady state and [S1] and [S2] represent substrate concentrations at steady state.

First we calculated the Gibbs energy change for GAPDH in *S. solfataricus* using the steady state substrates and products concentration values calculated by the model named 'S. solfataricus hy1'.

$$\Delta G(S.s : Pi = 10mM) = \Delta G^{0''} + RT \ln \frac{[BPG][NADPH]}{[GAP][NADP]} = \frac{17.9kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \times \ln \frac{[6.93 \times 10^{-6}][0.39]}{[0.15][1.2]} = -\frac{13.8kJ}{mol}$$

For the Gibbs energy change for PGK in *S. solfataricus* using the steady state substrates and products concentration values calculated by the model named 'S. solfataricus hy1'.

$$\Delta G(S.s : Pi = 10mM) = \Delta G^{0''} + RT \ln \frac{[3PG][ATP]}{[BPG][ADP]} = -\frac{23.5kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 343K \times \ln \frac{[0.0052][2.52]}{[6.93 \times 10^{-6}][1.32]} = -\frac{2.8kJ}{mol}$$

Then we calculated the Gibbs energy change for GAPDH in *S. cerevisiae* using the steady state substrates and products concentration values calculated by the model named 'S. cerevisiae hy1'.

$$\Delta G (S.c : Pi = 10mM) = \Delta G^{0''} + RT \ln \frac{[BPG][NADH]}{[GAP][NAD]} = \frac{8.374kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 303K \times \ln \frac{[0.007][0.39]}{[0.15][1.2]} = -\frac{2.2kJ}{mol}$$

Then we calculated the Gibbs energy change for PGK in *S. cerevisiae* using the steady state substrates and products concentration values calculated by the model named 'S. cerevisiae hy1'.

$$\Delta G (S.s : Pi = 10mM) = \Delta G^{0''} + RT \ln \frac{[3PG][ATP]}{[BPG][ADP]} = -\frac{20.31kJ}{mol} + \frac{8.314J}{mol \cdot K} \times 303K \times \ln \frac{[9.82][2.52]}{[0.007][1.32]} = -\frac{0.42kJ}{mol}$$